Saccharin consumption and the effect of a long-term exposure to a sweetened alcoholic solution in high- (UChB) and low- (UChA) alcohol-drinking rats

Lutske Tampier*, María Elena Quintanilla

Program of Molecular and Clinical Pharmacology, ICBM, Faculty of Medicine, University of Chile, P.O. Box 70.000, Santiago 7, Chile

Abstract

An association between saccharin consumption and alcohol intake has been observed in rodent lines genetically developed for alcohol preference or alcohol avoidance. It has also been proposed that a sweetened alcohol solution can condition rats to consume high amounts of alcohol. This work had two aims. First, to study the relationship between saccharin and alcohol intake in both high-alcohol-drinking UChB rats and low-alcohol-drinking UChA rats and, second, to determine whether a long-term exposure to a sweetened alcohol solution can increase their voluntary alcohol consumption. For the first purpose, UChB and UChA rats were tested under a free-choice paradigm between two graduated bottles, one containing a saccharin solution (0.1, 0.2, or 0.4% [wt/vol]) and the other water. For the second purpose, UChB and UChA rats that were under free choice between 10% alcohol and water, were offered a 10% alcohol solution containing 0.2% saccharin, instead of 10% alcohol for 1 month and were then returned to free choice between 10% alcohol and water. The first experiment showed that both lines have a high preference for saccharin at any concentration, but UChB rats drank twice as much saccharin solution as UChA rats and consequently they increased significantly their total daily fluid intake. This fact has been suggested to be an animal analogue of the clinical phenomenon known as ''loss of control.'' In the second experiment a long-term exposure to a 10% alcohol solution containing 0.2% saccharin induced a significant increase in alcohol consumption in UChB rats once saccharin was faded out, whereas the alcohol consumption in UChA rats returned to the previous low value. This result indicates that UChA rats have a genetic predisposition to avoid alcohol. In conclusion, the results reported here for UChB and UChA rats show an association between saccharin and alcohol preference, and suggest that their different genotypes are probably involved in alcohol aversion.

Keywords: UChA and UChB rats; Alcohol intake; Saccharin preference; Alcohol aversion

1. Introduction

A positive association between alcohol intake and consumption of sweet solutions has been observed in heterogeneous laboratory rodents (Overstreet et al., 1993) and also in humans (Kampov-Polevoy et al., 2003, 2004). An association between saccharin consumption and subsequent alcohol intake can be seen more clearly in rodent strains/lines genetically developed for alcohol preference or alcohol avoidance. For instance, selectively bred alcohol preferring AA (Alko, alcohol; Eriksson, 1968) and P (Indiana, Li et al., 1987) were found to consume significantly larger quantities of saccharin or sucrose solutions than their alcohol-avoiding counterparts, that is, ANA (Alko, nonalcohol) and NP, respectively (Kampov-Polevoy et al., 1992; Overstreet et al., 1993, 1997; Sinclair et al., 1992; Stewart et al., 1994). Moreover, in P rats, a significant increase in daily fluid intake (DFI) was observed when a saccharin solution was offered together with water (Kampov-Polevoy et al., 1995, 1999). This fact led the authors to suggest that the increase in DFI in the presence of a saccharin solution exhibited by P rats may be an animal analogue of the clinical phenomenon known as ''loss of control'' (Kampov-Polevoy et al., 1992, 1995). However, no association between alcohol intake and consumption of a sweet solution was observed in other lines of rats selectively bred for different consumption of alcohol, like the Sardinian alcohol-preferring and nonpreferring (sNP) rats (Colombo et al., 1995). Both of these ratlines showed a high degree of preference for a saccharin solution and only marginal differences were observed between the two lines, suggesting that their intake of saccharin is controlled by mechanisms that are different from those that control their alcohol intake (Agabio et al., 2000).

Additionally, it has been shown that long-term exposure to a sweetened alcohol solution conditions rats to consume

* Corresponding author. Tel.: +56-2-6786055; fax: +56-2-7372783.
E-mail address: ltampier@med.uchile.cl (L. Tampier).

high amounts of alcohol. Sweet-fading procedures have been proposed as methods of induction of high alcohol intake in laboratory animals (Gauvin et al., 1993). Specifically, a long-term exposure of NP rats to a sucrose-plus-alcohol solution resulted, when sucrose was faded out, in an alcohol intake that was equivalent to that recorded in the counterpart P rats (Gauvin et al., 1998). In contrast, a long-term exposure to a sweetened alcoholic solution did not alter the genetic aversion to alcohol in sNP rats (Brunetti et al., 2003).

Our high-alcohol-drinking UChB and low-alcohol-drinking UChA rats have been developed from Wistar rats at University of Chile (Mardones & Segovia-Riquelme, 1983). UChB high-alcohol-drinking rats consume voluntarily between 4 and 7 g alcohol·(kg body weight)$^{-1}$ · day, whereas UChA low-alcohol-drinking rats consume 0–2 g alcohol·(kg body weight)$^{-1}$·day^{-1}. They also differ genetically in their ALDH2 enzyme (Sapag et al., 2003). The low-alcohol-drinking UChA rats display a point mutation in the *Aldh2* gene as compared to the high-alcohol-drinking UChB rats. This *Aldh2* shows a low affinity (Km) for NAD that is four to fivefold higher than that of the *Aldh2* from UChB rats. Such a difference might explain the low-alcohol consumption of UChA rats: A lower rate of acetaldehyde metabolism would determine a genetic predisposition to avoid alcohol. On the other hand, wild type ALDH2 present in UChB rats predisposes them to increase their voluntary alcohol consumption when they are treated with an intraperitoneal dose of alcohol (2.3 g/kg) (Tampier & Quintanilla, 2002).

The purpose of this work was to address two questions. The first was to study whether the postulated relationship between saccharin and alcohol intake exists in UChB and UChA rats. The second was to determine whether the high-alcohol-drinking UChB rats and the low-alcohol-drinking UChA rats exposed to a sweetened alcoholic solution increase their voluntary alcohol consumption.

2. Materials and methods

2.1. Animals

UChB and UChA rats (bred at the Faculty of Medicine, University of Chile) (Mardones & Segovia-Riquelme, 1983) of both genders and 3–4 months old were used throughout the experiments. Rats were housed in individual cages in a room regulated at 22 ± 2°C on a regular 12-h light/12-h dark cycle and fed ad libitum on a balanced diet lacking animal products.

2.2. Experiment 1

Ten UChB (five ♀ and five ♂) and 10 UChA (five ♀ and five ♂) experimentally naïve rats were tested with a free choice between two graduated bottles, one containing distilled water and the other a solution of increasing saccharin concentration (0.1%, 0.2%, and 0.4% wt/vol saccharin in water) under an unlimited access paradigm for 6 consecutive days with each saccharin concentration. Saccharin and water intakes were monitored daily. Bottles were refilled every day with fresh solution.

2.3. Experiment 2

Ten UChB (five ♀ and five ♂) and 10 UChA (five ♀ and five ♂) rats under the paradigm of the two-bottle free choice were offered distilled water and a 10% vol/vol alcohol solution for 2 months. Daily alcohol consumption during the last 30 days was averaged to obtain the mean alcohol consumption for each animal (phase 1). On the third month, rats were offered a 10% alcohol solution sweetened with 0.2% saccharin and water ad libitum (phase 2). Alcohol containing 0.2% saccharin and water intakes were monitored daily. Finally, rats were tested for preference between unsweetened alcohol and water for 30 additional days (phase 3), immediately after the preceding phase with no interruption. Daily alcohol and water intakes during each phase of 30 days were averaged every 5 days.

Alcohol consumption was expressed as ml/kg body weight. Daily water consumption was expressed as ml/kg body weight and total DFI as the sum of water and 0.9 × 10% alcohol. Preference for saccharin was expressed as percentage of saccharin solution consumed over total DFI.

2.4. Statistical analysis

Results are expressed as the mean ± standard error of the mean. M. In experiment 1, a mixed-model analysis of variance with two between-subjects factors (line and gender) and two within-subjects factors (saccharin concentration [0.1, 0.2, and 0.4%] and day [6 days each period]) was used to evaluate saccharin consumption and total fluid intake of the two selected lines of rats. In experiment 2, the generalized estimating equation population-averaged model was used to address the interaction of saccharin intake on the 10% alcohol consumption by both lines of rats.

3. Results

3.1. Experiment 1

As shown in Fig. 1, UChB and UChA rats consumed large quantities of saccharin, and in both lines of rats the mean intake of saccharin was consistently higher than water intake. Thus, both lines of rats presented a high preference for a saccharin solution but saccharin intake in UChB rats doubled that of UChA rats. Saccharin preference expressed as percentage of saccharin solution consumed over total fluid intake was 97% for UChB and 85% for UChA rats. No gender difference was observed in saccharin or total fluid intake. The mixed-model ANOVA on saccharin

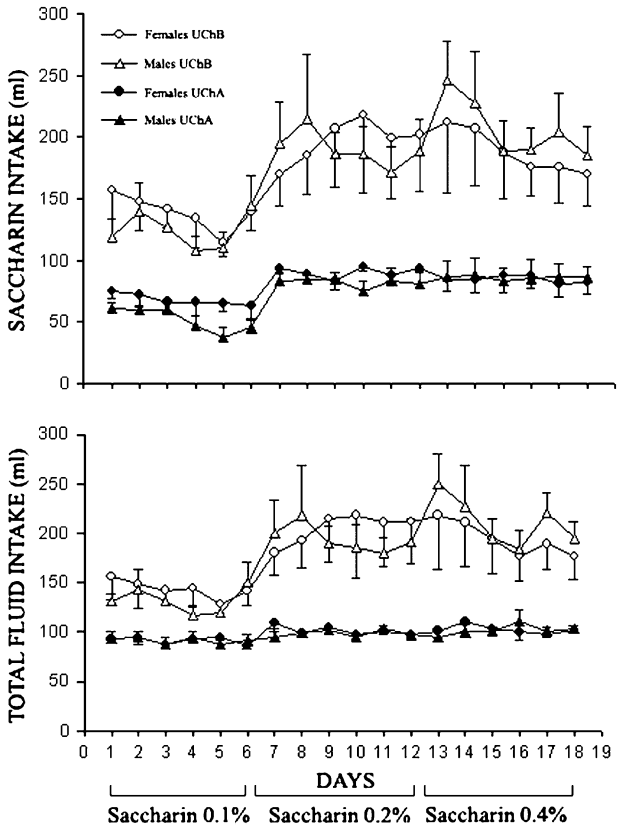

Fig. 1. Daily intake of increasing saccharin concentration (0.1%, 0.2%, 0.4% solution) and total daily fluid intake (DFI) under an unlimited access paradigm for 24 h/day for 6 consecutive days with each saccharin concentration in alcohol-naïve high drinker UChB (○) and low drinker UChA (●) rats. Saccharin intake and DFI are expressed in ml/kg body weight. Each point is the mean ± standard error of the mean of 10 rats.

intake showed significant effects of line [$F(1, 353) = 185.05$, $P < .0001$], gender [$F(1, 353) = 48.10$, $P < .0001$], and saccharin concentration [$F(2, 353) = 38.29$, $P < .0001$]; there was no significant effect of day. A post hoc analysis revealed that the mean daily intake of the different saccharin concentrations was significantly higher in UChB rats than in the UChA rats ($P < .001$). Also, a significant difference in saccharin consumption was observed in UChB rats when consuming 1% saccharin than when consuming 2% saccharin ($P < .001$) and between 1% and 4% saccharin ($P < .001$). For UChA rats, a significant difference was observed between saccharin 1% versus 2% ($P < .001$) and 1% versus 4% ($P < .001$).

Concomitantly, the mixed-model ANOVA on total fluid intake showed significant effects of line [$F(1, 353) = 131.33$, $P < .0001$], gender [$F(1, 353) = 33.79$, $P < .0001$], and saccharin concentration [$F(2, 353) = 27.83$, $P < .0001$], but no effect of day. The mean daily total fluid intake was significantly higher in UChB rats than in the UChA rats ($P < .001$). In UChB rats, a significant difference was evident between total fluid intake when consuming 1% saccharin than when consuming 2% saccharin ($P < .001$), and also between 1% and 4% saccharin ($P < .001$). For UChA rats, no significant difference existed for total fluid intake across the different saccharin concentrations.

The daily voluntary alcohol consumption under the two-bottle choice paradigm after the saccharin experiments was 6.2 ± 0.6 g alcohol·(kg body weight)$^{-1}$·day^{-1} for UChB rats and 0.3 ± 0.1 g alcohol·(kg body weight)$^{-1}$·day^{-1} for UChA rats.

3.2. Experiment 2

Fig. 2 shows the daily intake of alcohol and total DFI for both lines of rats before (phase 1), during (phase 2), and after (phase 3) 1 month of consuming a 10% (vol/vol) alcohol solution containing 0.2% (wt/vol) saccharin (the experimental condition). The analysis of data depicted in Fig. 2 by the generalized estimating equation population-averaged model showed that UChB rats having free access to a sweetened alcohol solution and water (phase 2) increased their

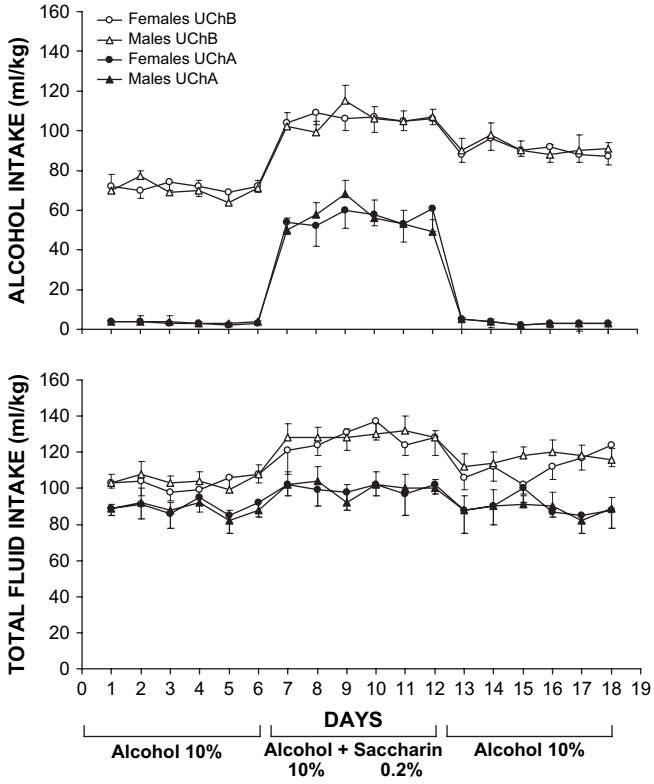

Fig. 2. Daily 10% alcohol intake and total daily fluid intake (DFI) in high drinker UChB (○) and low drinker UChA (●) rats before, and after the experimental addition of saccharin (2 g/L) to the alcohol solution. Alcohol intake occurred under the two-bottle free-choice regimen with water for 24 h/day. First, rats were offered 10% alcohol solution for 30 consecutive days (phase 1), then saccharin (2 g/L) was added to 10% alcohol solution during next 30 days (phase 2), afterward saccharin was faded out from 10% alcohol solution for other 30 consecutive days (phase 3). No interruption was interposed between phases. Alcohol intake and DFI are expressed in ml/kg body weight. Each point is the mean ± standard error of the mean of the average consumption of 10 rats every 5 days during each phase.

alcohol consumption significantly when compared to the amounts that these rats drank before the experimental condition (phase 1) ($z = 7.41$, $P < .001$). When UChB rats were returned to the free choice between water and a 10% alcohol solution (phase 3), they maintained their characteristic high alcohol intake but significantly lower than that during phase 2 ($z = -3.41$, $P < .001$) yet higher than the amount of alcohol that these rats drank under the same condition during phase 1 ($z = 4.00$, $P < .001$). Total DFI of UChB rats also increased after 1 month of access to alcohol solution containing 0.2% saccharin (phase 2) as compared to that seen in phase 1 ($z = 4.4$, $P < .001$). When these rats were returned to the free choice between water and 10% alcohol solution (phase 3), they maintained the high DFI that was seen in phase 2 ($z = -1.83$, $P < .067$), which was significantly higher than that seen in phase 1 ($z = 2.57$, $P < .001$).

UChA rats given access to a 10% alcohol solution containing 0.2% saccharin and water (phase 2) also increased their alcohol consumption significantly ($z = 10.60$, $P < .001$). However, when saccharin was faded out from the 10% alcohol solution (phase 3) UChA rats drank significantly lower amounts ($z = -10.66$, $P < .001$), returning to their characteristically low-alcohol consumption. Phase 3 alcohol consumption was not significantly different from that of phase 1 ($z = 0.06$, $P < .951$) for UChA rats. No significant differences were observed in DFI across phases [phase 2 vs. phase 1 ($z = 0.24$, $P < .809$); phase 2 vs. phase 3 ($z = 0.19$, $P < .851$); phase 3 vs. phase 1 ($z = 0.05$, $P < .957$)].

4. Discussion

The data from the saccharin experiments indicate that both UChB and UChA rats show a high propensity toward consumption of the saccharin solution at any of the concentrations. However, UChB rats drink significantly more saccharin solution than UChA rats under the same condition over the range of saccharin concentrations studied here (Fig. 1). The results support a close relationship between genetic factors influencing alcohol and saccharin intake in both ratlines.

One of the interesting characteristics of saccharin intake of P rats is their tendency to consume saccharin beyond the limit of normal DFI (Kampov-Polevoy et al., 1995, 1999). Rats genetically selected for alcohol preference show an even greater saccharin-induced polydipsia. For example, high-alcohol-preferring rats exhibited a 370% increase in DFI when a saccharin solution was available along with water, whereas low-alcohol-preferring rats consumed saccharin solution within their normal DFI (Overstreet et al., 1997). Kampov-Polevoy et al. (1995) suggested that the increase of DFI in the presence of a saccharin solution exhibited by P rats may be an animal analogue of the clinical phenomenon known as ''loss of control.'' In the clinical situation, loss of control refers to the behavior in which a rewarding substance is taken in larger amounts or over longer periods of time than is intended. P animals tend to choose more concentrated sweet solutions compared to NP animals. Similar tendencies were noted in studies of alcoholic and nonalcoholic subjects, with most alcoholics preferring sweeter sucrose solutions (Kampov-Polevoy et al., 1997). Our high-alcohol-drinking UChB rats also show a substantial increase in their DFI when saccharin is present (Fig. 1). A significant increase in DFI was observed only in UChB rats under free choice between saccharin solution and water, whereas under the same condition the low-alcohol-drinking UChA rats consumed the saccharin solution within the limits of their DFI.

Although the mechanism of association between consumption of sweet solutions and alcohol intake is not fully understood, it may be speculated that it is determined by a common mechanism mediating the rewarding properties of both sweet and alcohol solutions. It has been shown that various drugs of abuse and sweet foods share the ability to increase the extracellular concentration of dopamine in the nucleus accumbens (Di Chiara et al., 1998; Hajnal et al., 2004) suggesting that alcohol and sweets may share a common dopaminergic mechanism in mediating their hedonic effects. Previous results showing that sulpiride (a D2 receptor antagonist) decreases alcohol consumption in UChB rats (Mardones et al., 1984) support this notion. Taken together, these results suggest that this relationship might be related to similar mechanisms mediating the reinforcement from sweet taste and from systemic alcohol.

Alcohol consumption by rats appears to be learned, the preference for alcohol needs to be developed and in most cases, to be enhanced by prolonged and/or forced exposure to alcohol (Adams et al., 1991; Waller et al., 1982). It has been proposed that selectively bred NP rats can ''learn'' to consume alcohol in amounts equivalent to those consumed by P rats when ethanol is offered in palatable fluids and over prolonged periods of time (Gauvin et al., 1998). In effect, when NP rats were exposed for a long term to a sucrose alcoholic solution, and then sucrose was faded out, alcohol intake in NP rats was equivalent to that recorded in P rats, indicating that NP rats could be conditioned to consume amounts of alcohol similar to those consumed by P rats. In contrast, long-term exposure to a sweetened alcohol solution did not alter the genetic aversion to alcohol in sNP rats (Brunetti et al., 2003). Results of the exposure to a 10% alcohol solution containing a highly accepted concentration of saccharin show that this treatment also failed to induce any relevant alcohol-drinking behavior in our low-alcohol-drinking UChA rats. Although alcohol consumption in UChA rats having access to a sweetened alcohol solution increases with respect to the consumption they exhibited under free choice of 10% alcohol solution and water (4.4 ± 0.8 vs. 0.3 ± 0.2 g alcohol$\cdot$kg$^{-1}\cdot$day^{-1}, respectively), alcohol consumption appeared to be maintained predominantly by saccharin intake because alcohol drinking was dramatically

reduced once saccharin was faded out and rats were returned again to free choice between water and 10% ethanol solution (Fig. 2). Results of the current study indicate that a long exposure to a sweetened alcohol solution failed to overcome the genetic predisposition to avoid alcohol in UChA rats. Moreover, UChA rats did not increase their alcohol intake after a long-term forced exposure to a 10% alcohol solution (L. Tampier & M. E. Quintanilla, unpublished observation). In contrast to NP rats, these results indicate that the mechanism underlying the genetic predisposition to avoid alcohol in UChA rats is different from that involved in other alcohol-avoiding rats from different foundation stocks. We postulate that the different genotype of ALDH2 present in UChA rats is involved in the development of alcohol aversion (Quintanilla et al., 2005) inducing a greater sensitivity of UChA rats to the aversive than to the rewarding attributes of alcohol (Quintanilla et al., 2001).

On the contrary, the free access of high-alcohol-drinking UChB rats to a sweetened alcohol solution leads to a significant increase in their alcohol consumption when compared to their previous alcohol consumption under free choice between a 10% alcohol solution and water, 8.5 ± 0.6 versus 5.7 ± 0.4 g alcohol$\cdot$kg$^{-1}\cdot$day^{-1}, respectively. When saccharin is faded out, UChB rats maintain the increase in alcohol consumption that they had when alcohol contained 0.2% saccharin. These results lead us to suggest that the high-alcohol-drinking UChB rats may have a greater sensitivity to the rewarding attributes of alcohol, because they increase significantly their alcohol intakes after a free choice between water and 10% alcohol solution containing 0.2% saccharin. The results also support the suggestion that while having access to a 10% alcohol solution containing 0.2% saccharin and water for 1 month, UChB rats develop tolerance to the aversive effects of alcohol or a higher sensitivity to the positively hedonic properties of alcohol. In accordance with this, previous results show that UChB rats can increase their voluntary alcohol consumption when they are treated with an IP dose of alcohol (Tampier & Quintanilla, 2002) or if they are forced to drink when offered alcohol only (L. Tampier & M. E. Quintanilla, unpublished observation). This may be explained because UChB rats can develop acute tolerance to a dose of alcohol (Tampier & Mardones, 1999). In summary, these results also suggest that saccharin and alcohol could be operating directly on one or more of the same neurochemical systems underlying the reinforcing effects of these agents, alcohol being more rewarding because rats do not need to increase their DFI to reach their hedonic set-point mechanism that regulates their alcohol intake (Agabio et al., 2000). It is generally accepted that tolerance and sensitization are analogous to a learning process, because they involve an adaptive change in response to stimulation (Goudie & Demelweek, 1986).

The results here reported also show an association between saccharin and alcohol intake as has been reported by several investigators in other P and NP rats (Kampov-Polevoy et al., 1992; Overstreet et al., 1993, 1997; Sinclair et al., 1992; Stewart et al., 1994). Additionally, these results suggest that saccharin and alcohol preference may have a similar genetic basis, whereas different genotypes are probably involved in the development of alcohol aversion and that the contribution of each genotype may vary between different alcohol-avoiding rats.

Acknowledgments

The authors thank Dr. Amalia Sapag for language revision, Prof. Jorge Rodriguez for the statistical analysis of the data, and Juan Santibañez for animal breeding and care.

References

Adams, N., Shihabi, Z. K., & Blizard, D. A. (1991). Ethanol preference in the Harrington derivation of the Maudsley reactive and nonreactive strains. *Alcohol Clin Exp Res 15*, 170–174.

Agabio, R., Carai, M., Lobina, C., Pani, M., Reali, R., Bourov, I., Gessa, G. L., & Colombo, G. (2000). Dissociation of ethanol and saccharin preference in sP and sNP rats. *Alcohol Clin Exp Res 24*, 24–29.

Brunetti, G., Serra, S., Vacca, G., Lobina, C., Curreli, T., Carai, M. A., Colombo, G., & Gessa, G. L. (2003). Long-term exposure to a sweetened alcoholic solution does not alter genetic aversion to ethanol in Sardinian alcohol-nonpreferring (sNP) rats. *Alcohol 30*, 29–34.

Colombo, G., Agabio, R., Lobina, C., Reali, R., Zocchi, A., Fadda, F., & Gessa, G. L. (1995). Sardinian alcohol-preferring rats: a genetic model of anxiety. *Physiol Behav 57*, 1181–1185.

Di Chiara, G., Tanda, G., Cadoni, C., Acquas, E., Bassareo, V., & Carboni, E. (1998). Homologies and differences in the action of drugs of abuse and conventional reinforcer (food) on dopamine transmission: an interactive framework of the mechanism of drug dependence. *Adv Pharmacol 42*, 983–987.

Eriksson, K. (1968). Genetic selection for voluntary alcohol consumption in the albino rat. *Science 159*, 739–741.

Gauvin, D. V., Moore, K. R., & Holloway, F. A. (1993). Do rat strain differences in ethanol consumption reflect differences in ethanol sensitivity or the preparedness to learn? *Alcohol 10*, 37–43.

Gauvin, D. V., Vanecek, S. A., Baird, R. J., Briscoe, R. J., Vallett, M., & Holloway, F. A. (1998). Genetic selection of alcohol preference can be countered by conditioning processes. *Alcohol 15*, 199–206.

Goudie, A. J., & Demelweek, C. (1986). Conditioning factors in drug tolerance, In S. R. Goldberg, & I. P. Stoleman (Eds.), *Behavioral Analysis of Drug Dependence* (pp. 225–285). New York: Academic Press.

Hajnal, A., Smith, G. P., & Norgren, R. (2004). Oral sucrose stimulation increases accumbens dopamine in the rat. *Am J Physiol Regul Integr Comp Physiol 286*, 31–37.

Kampov-Polevoy, A. B., Eick, C., Boland, G., Khalitov, E., & Crews, F. T. (2004). Sweet liking, novelty seeking, and gender predict alcoholic status. *Alcohol Clin Exp Res 28*, 1291–1298.

Kampov-Polevoy, A. B., Garbutt, J. C., & Janowsky, D. S. (1997). Evidence of preference for a higher concentration sucrose solution in alcoholic men. *Am J Psychiatry 154*, 269–270.

Kampov-Polevoy, A. B., Garbutt, J. C., & Janowsky, D. S. (1999). Association between preference for sweets and excessive alcohol intake: review of animals and human studies. *Alcohol Alcohol 34*, 386–395.

Kampov-Polevoy, A. B., Garbutt, J. C., & Klalitov, E. (2003). Family history of alcoholism and responses to sweets. *Alcohol Clin Exp Res 27*, 1743–1749.

Kampov-Polevoy, A. B., Overstreet, D. H., Rezvani, A. H., & Janowsky, D. S. (1995). Saccharin-induced increase in daily fluid intake as a predictor of voluntary alcohol intake in alcohol-preferring rats. *Physiol Behav 57*, 791–795.

Kampov-Polevoy, A. B., Overstreet, D. H., Rezvani, A. H., Janowsky, D. S., & Halikas, J. A. (1992). Taste sensitivity and alcohol preference in preferring and nonpreferring strains of rats. *Alcohol Clin Exp Res 16*, 405.

Li, T. K., Lumeng, L., McBride, W. J., & Murphy, J. M. (1987). Rodent lines selected for factors affecting alcohol consumption. *Alcohol Alcohol 1*, 91–96.

Mardones, J., & Segovia-Riquelme, N. (1983). Thirty-two years of selection of rats by ethanol preference: UChA and UChB strains. *Neurobehav Toxicol Teratol 5*, 171–178.

Mardones, J., Segovia-Riquelme, N., & Contreras, S. (1984). Effects of sulpiride on the voluntary consumption of ethanol, water, and solid food by UChA and UChB rats. *IRCS Med Sci 10*, 375–376.

Overstreet, D. H., Halikas, J. A., Seredenin, S. B., Kampov-Polevoy, A. B., Viglinskaya, I. V., Kashevskaya, O., Badishtov, B. A., Knapp, D. J., Mormede, P., Kiianmaa, K., Li, T. K., & Rezvani, A. (1997). Behavioural similarities and differences among alcohol-preferring and -nonpreferring rats: confirmation by factor analysis and extension to additional groups. *Alcohol Clin Exp Res 21*, 840–848.

Overstreet, D. H., Kampov-Polevoy, A. B., Rezvani, A. H., Murrelle, L., Halikas, J. A., & Janowsky, D. S. (1993). Saccharin intake predicts ethanol intake in genetically heterogeneous rats as well as different rats strains. *Alcohol Clin Exp Res 17*, 366–369.

Quintanilla, M. E., Callejas, O., & Tampier, L. (2001). Differences in sensitivity to the aversive effects of ethanol in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. *Alcohol 23*, 177–182.

Quintanilla, M. E., Tampier, L., Sapag, A., & Israel, Y. (2005). Polymorphisms in the mitochondrial aldehyde dehydrogenase gene (*ALdh2*) determine peak blood acetaldehyde levels and voluntary ethanol consumption in rats. *Pharmacogenet Genomics 15*, 427–431.

Sapag, A., Tampier, L., Valle-Prieto, A., Quintanilla, M. E., Moncada, C., & Israel, Y. (2003). Mutations in mitochondrial aldehyde dehydrogenase (AlDH2) change cofactor affinity and segregate with voluntary alcohol consumption in rats. *Pharmacogenetics 13*, 509–515.

Sinclair, J. D., Kampov-Polevoy, A., Stewart, R., & Li, T. K. (1992). Taste preferences in rat lines selected for low and high alcohol consumption. *Alcohol 9*, 155–160.

Stewart, R. B., Russell, R. N., Lumeng, L., Li, T. K., & Murphy, J. M. (1994). Consumption of sweet, salty, sour and bitter solutions by selectively bred alcohol-preferring and alcohol-nonpreferring lines of rats. *Alcohol Clin Exp Res 18*, 375–381.

Tampier, L., & Mardones, J. (1999). Differences in ethanol sensitivity and acute tolerance between UChA and UChB rats. *J Stud Alcohol 60*, 168–171.

Tampier, L., & Quintanilla, M. E. (2002). Effect of a dose of ethanol on acute tolerance and ethanol consumption in alcohol drinker (UChB) and non-drinker (UChA) rats. *Addict Biol 7*, 279–284.

Waller, M. B., McBride, W. L., Lumeng, L., & Li, T. K. (1982). Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats. *Pharmacol Biochem Behav 16*, 501–507.